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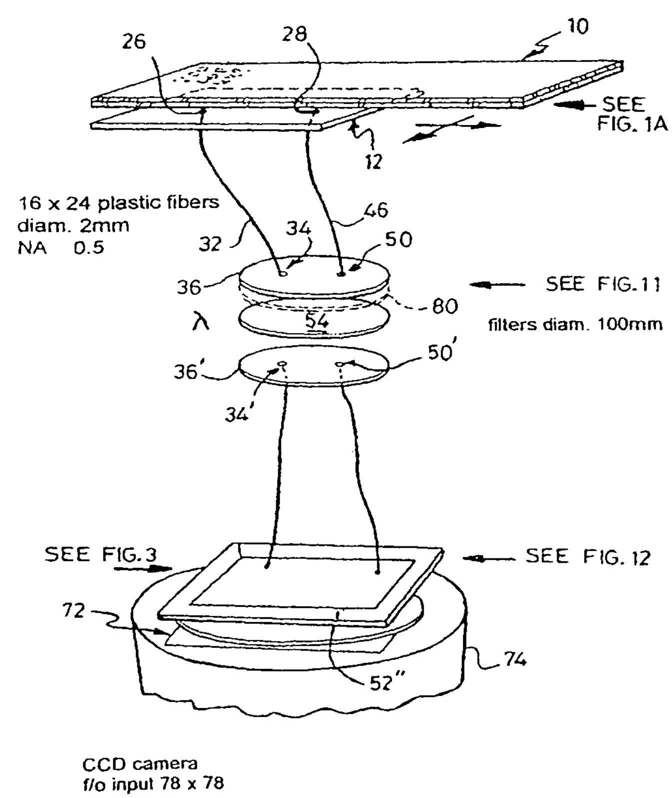
(54) Title: LUMINESCENCE IMAGER



(57) Abstract: A fibre optic epi-fluorescence imaging system in which the optical fibres are rearranged so that the system can be used for measuring luminescence samples. The system comprises at least two optical fibres (32, 46) or bundles of fibres which lead to a CCD camera (74), the fibres or bundles of fibres from all samples being arranged in two sets, a first set which are formed from a non-fluorescing material and a second set which are formed from a material which may fluoresce but enables the fibres formed therefrom to have a higher numerical aperature than those of the first set.

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Title: Luminescence Imager

Field of Invention

This invention concerns luminescence imaging methods and apparatus, particularly methods and apparatus which employ optical fibres and a CCD to inspect and monitor luminescence from arrays such as microtitre plates.

Background

Reference is made to Patent Specifications GB 2339900, GB 2339901, and GB 2351555, which describe an epifluorescence imaging system designed to measure the fluorescence emitted from the bottom of an array of samples when they are irradiated from beneath by excitation light. Figure 1, taken from these Applications, shows the use of an array of typically 8 x 12 trifurcated fibre optic bundles arrayed in a presentation plate on which the sample plate is placed. One sub-set of fibres within a bundle is used to bring in the excitation light, and two other sub-sets of fibres in each bundle collect emitted fluorescent light into two separate filter channels to enable simultaneous measurement of dual wavelengths. After the filters the light is transmitted through single fibres, 2 x 96 of them, to a fibre optic input face of a CCD camera. The arrangement of optical fibre bundles at the filters is shown in Figure 2, and of the optical fibres at the input to the CCD camera is shown in Figure 3. Figure 4 shows how the different categories of fibres are arranged in a hexagonal pattern within a bundle.

Typically an image can be taken every second, and repeated at approximately one-second intervals.

This system has proved to be an efficient, highly sensitive measurer of fluorescence in dual wavelengths, and the ability to measure dual wavelengths simultaneously is of great value for certain assays, such as FRET assays, where a change in the ratio of the signal strengths in the two wavelengths is an indicator of chemical binding or some other attribute of the assay.

This 96-channel system can be used to measure 96-well microtitre plates, or by stepping the microtitre plate over the presentation plate, larger assay presentations such as 384, 864, 1536, 3465...., well plates (i.e. having and n^2*96 pattern, where n is an integer).

This system is not optimised for the measurement of luminescence for which no excitation is needed, and in which a sample emits light by virtue of a chemical reaction occurring when reagents are interacting in the sample well.

Thus, for fluorescence, the fibre bundle is stood off below the well since light has to illuminate the sample evenly and receive fluorescence light with uniform efficiency from all parts of the well. This still applies even if the sample is a cellular layer on the base of the well.

Therefore although this epi-fluorescence system can be used to measure luminescence, it is not optimal, and is generally sensitive enough only for the brightest types of samples and assays. Examples of such assays include detection using luciferase enzyme; calcium detection, for example in cells, linked to the enzyme aequorin, which produces a flash of light during the reaction; and alkaline phosphatase linked assays using enhanced chemiluminescence substrates.

Summary of the Invention

According to one aspect of the present invention an imaging system such as has been proposed for use with epi-fluorescence may be adapted for use with luminescence samples

(which in general produce less light than epi-fluorescence samples) by optimising the position and type of fibre optic read out.

In one embodiment of the invention it is proposed to use a second presentation plate in which, say, 8 x 12 single fibres are mounted, each with its face immediately below or in contact with the base of the sample plate. The end face of each fibre which is to view the plate is preferably polished. Typically the fibres are optical fibres of plastics fibre material, say 2mm diameter, which is available at low cost with relatively high numerical aperture (NA) - e.g. NA = 0.5. This is to be compared with the NA (typically about 0.2) of the silica fibres which are commonly used to make the fibre bundles for an epi-fluorescence system. Silica fibre material is used to minimise the auto-fluorescence background that can occur in the presence of excitation light. The use of plastics material for fibres in fluorescence systems is ruled out since plastics materials tend to fluoresce.

Using such an arrangement of plastics fibres, a factor of light gathering improvement of the order of 10-20 has been obtained for luminescence samples when compared with the measurement of these samples using an epi-fluorescence system. This factor results both from the closer placing of the end of each fibre to the sample, and from the higher NA of the fibre. This makes possible the measurement of weaker types of luminescence.

According to a second aspect of the invention an epi-fluorescence imaging system may be adapted for use with luminescence samples by leading a second set of high NA fibres to the input of the CCD to which the fibres employed for epi-fluorescence samples are also led, the two sets of fibres being arranged interstitially, so that a second CCD camera is not needed.

Although the combined system will be used either for fluorescence or for luminescence, there is no risk of cross talk between the closely spaced fibres, since only one set at a time is illuminated.

According to a further aspect of the invention the luminescence light can be analysed in two wavelengths simultaneously by means of a bifurcation of each of the fibres employed when looking for luminescence. Thereafter there would be two parallel filter channels, and the two sets of fibres would be mounted interstitially to the two sets of fluorescence transfer fibres.

Certain types of luminescence assays benefit from analysis at two wavelengths. Examples of this include multi-labelled assay systems, where more than one label has been used in an assay. A further example is resonant energy transfer type assays, where a donor molecule emits light at one wavelength and a receptor molecule emits light at a second wavelength. The degree of energy transfer between the two molecules is measured by the ratio of the intensity of the two wavelengths emitted by the molecules.

The analysis algorithms already proposed (such as referred to in the Patent Specifications listed earlier) for performing measurements on fluorescence derived signals can be readily extended to luminescence signals from the CCD camera. Thus for example in the case of a 96-fibre array, a set of geometrically fixed 96 spots of light (or 2 x 96 spots) can be registered at a fixed displacement on the CCD from the 2 x 96 fluorescence spots.

A fixed array of fibres mounted in a presentation plate for luminescence measurements can be used independently of the epifluorescence system. An example would be a 16 x 24 fibre array to measure 384 channels simultaneously.

The fibres in the filter region may be arranged on a 100mm-diameter filter and the system would require its own CCD camera, but this would be the only significant additional element.

The 384 fibres at the input to the CCD camera may be re-arranged if required to accommodate the aspect ratio of the CCD.

Such an arrangement can also measure a 1536 well, plate by means of a 2 x 2 stepping of the sample plate relative to the presentation plate.

As before, each fibre can be bifurcated to enable dual wavelength analysis. This system represents a low-cost, optically efficient means of measuring luminescence in larger size well plates, (384, 1536 wells or more).

In an arrangement embodying the invention for luminescence samples, a fixed fibre array for the measurement of arrays of samples has the following advantages over a lens based method.

(1) absence of parallax (so that wells in the corners of a plate are measured as efficiently as those in the centre of the plate) which is only avoided by using an (expensive) telecentric lens;

(2) no vignetting;

(3) no need to focus:

(4) there is much more efficient light gathering than from a lens.

Thus an optical fibre placed immediately under a well of about the same diameter has optical efficiency approximately equal to the $(NA)^2 = (0.5)^2 = 0.25$. On the other hand a quality telecentric lens (say $F=1$) imaging a whole plate (size 110 x 75mm) onto a CCD (size 25 x 25 mm) so that the lens demagnification is $m=110/25 = 4.4$, has optical efficiency $1/\{2F(1+m)\}^2 = 0.0086$.

A set of optical fibres as proposed is therefore about 30 times more efficient, and much less expensive than a quality lens.

The advantages of the invention are achieved if the end of each fibre that is to collect light from a sample is very close to, if not touching, the sample, either above or below the sample, such as a well in a multi-well plate, for example.

The invention also lies in a system for inspecting light emitting samples such as are contained in a well plate from each of which at least two optical fibres or bundles of fibres lead to a CCD camera, the fibres or bundles of fibres from all samples being arranged in two sets, a first set which are formed from a non-fluorescing material and a second set which are formed from a material which may fluoresce but enables the fibres formed therefrom to have a higher numerical aperture than those of the first set, one of the said at least two fibres linked to each sample belonging to the one set, and the other to the other set, wherein the fibres are arranged interstitially at the input to the camera, the light emitted from each fibre falling on a uniquely addressable region of the CCD, and the addressing of the CCD is arranged so that the addresses associated with one set of fibres, or the other, are read out and refreshed to produce an output signal for analysis.

By reading out the addresses relating to the first set of fibres, an output signal is obtained relating to any fluorescence from the samples.

By reading out the addresses relating to the second set of fibres an output signal will relate to any luminescence produced by the samples.

Where the samples need to be excited so as to produce fluorescence, additional fibres lead to the samples from an excitation source.

Additional sets of fibres, each set illuminating a different unique set of addresses of the CCD, may be provided with appropriate wavelength selection as by filtering, to enable dual wavelength analysis to be performed on light emitting samples.

Using bi-furcated fibres in the second set, so as to produce 2N fibres leading from N wells, with one set of N leading to the CCD input, may allow the other set to be used for

transmitting excitation radiation from a suitable source to the samples, thereby obviating the need for a third set of N fibres to the wells, for conveying excitation radiation thereto.

The invention is illustrated by way of example in Figs 5 onwards.

The invention extends the current epi-fluorescence system into a whole new application, namely detection of luminescence, at a minimal cost, and in a technically simple manner. As before, microtitre plates having 384, 864, 1536....wells, based on a 96-channel pattern, can be measures by stepping the sample plate over the presentation plate. Figure 5 shows a 96-channel luminescence reading head.

A filter can be added to this luminescence system, as shown in Figure 7, which needs the same arrangement of 96 extra fibres at the CCD shown in Figure 6.

The arrangement of fibres in the filter space would be as in Figure 2. As with the fluorescence version, there is a rearrangement of the fibres from the sample presentation plate to the filter space, if a filter is used, and again to the input to the CCD camera. Note that the filter space does not necessarily need to be as thick as in the fluorescence version, as there is no excitation light that has to be blocked from entering the emission path, allowing simpler, thinner filters to be used for the luminescence filter space.

Fig 6 shows the interstitial arrangement of the fibres to be used for transmitting luminescence light to the camera, with those of the known epi-fluorescence system. All the fibres lead to the one entrance to the CCD camera.

Bifurcation of the luminescence fibres is shown in Fig 8.

Interstitial placement of the two sets of fibres is again shown in Fig 9.

Fig 10 shows an example of a 16 x 24 fibre array, which allows for simultaneous inspection of all the wells in a 384 well plate. As shown in Fig 11, the 384 fibres are

arranged on a 100mm diameter filter where they extend through the filter space of the epi-fluorescence system.

Fig 12 shows the arrangement of the 384 fibres at the CCD camera input.

CLAIMS

1. An imaging system for measuring luminescence produced by an array of samples in a sample plate, wherein a presentation plate is located immediately adjacent or in contact with the sample plate, this presentation plate presenting the ends of a matching array of optical fibres to at least part of the array of samples, each such fibre having a numerical aperture (NA) greater than 0.2, and the said array of optical fibres conveying luminescence emitted by the samples to a CCD camera.

2. A system according to claim 1, wherein the optical fibres have a NA of about 0.5.

3. A system according to claim 1 or claim 2, wherein the optical fibres are made of plastics material.

4. A system according to any of claims 1 to 3, wherein the ends of the optical fibres viewing the sample plate are polished.

5. A system for alternatively measuring epi-fluorescence or luminescent produced by samples in a sample plate, wherein for measuring eip-fluorescence a presentation plate having an array of silica optical fibres of NA of about 0.2 or less is presented to the sample plate at a small spacing therefrom and for measuring luminescence a presentation plate according to the system of any of claims 1 to 4 is presented to the sample plate.

6. A system according to claim 5, wherein, at the CCD camera, the lower NA fibres for conveying epi-fluorescence are arranged interstitially with the higher NA fibres for conveying luminescence, so that a second CCD camera is not required, an excitation source for epi-fluorescence measurements being switched off during luminescence measurements.

7. A system according to any of claims 1 to 6, wherein the fibres for conveying luminescence are bifurcated to enable the luminescence to be conveyed to the CCD by fibre subsets including two parallel filter channels.

8. A system acording to claim 7 when appended to claim 5, wherein the fibres for conveying epi-fluorescence are also arranged in subsets including two filter channels, and the two luminescence subsets are mounted interstitially to the two epi-fluorescence subsets.

9. A system according to any of claims 1 to 8, wherein the fibres are re-arranged at the CCD camera to accommodate the aspect ratio of said camera.

10. A system according to any of claims 1 to 9, wherein an analysis algorithm is employed to measure the luminescence signals received at the CCD camera, said algorithm being a modification of a known algorithm for measuring epi-fluorescence signals.

11. A system according to any of claims 1 to 10, wherein, when the presentation plate is of a size smaller than the sample plate, the sample plate and the presentation plate are relatively moved to enable luminescence at different groups of samples to be measured in succession.

12. A system for inspecting light emitting samples such as are contained in a well plate from each of which at least two optical fibres or bundles of fibres lead to a CCD camera, the fibres or bundles of fibres from all samples being arranged in two sets, a first set which are formed from a non-fluorescing material and a second set which are formed from a material which may fluoresce but enables the fibres formed therefrom to have a higher numerical aperture than those of the first set, one of the said at least two fibres linked to each sample belonging to the one set, and the other to the other set, wherein the fibres are arranged interstitially at the input to the camera, the light emitted from each fibre falling on a uniquely addressable region of the CCD, and the addressing of the CCD is arranged so that the addresses associated with one set of fibres, or the other, are read out and refreshed to produce an output signal for analysis.

13. A system according to claim 12, wherein, by reading out the addresses relating to the first set of fibres, an output signal is obtained relating to any fluorescence from the samples, and by reading out the addresses relating to the second set of fibres an output signal is obtained relating to any luminescence produced by the samples.

14. A system according to claim 12 or claim 13, wherein, when the samples need to be excited so as to produce fluorescence, additional fibres lead to the samples from an excitation source.

15. A system according to any of claims 12 to 14, wherein additional sets of fibres, each set illuminating a different unique set of addresses of the CCD, may be provided with appropriate wavelength selection as by filtering, to enable dual wavelength analysis to be performed on light emitting samples.

16. A system according to any of claims 12 to 15, wherein bi-furcated fibres are used in the second set, so as to produce 2N fibres leading from N wells, with one set of N leading to the CCD input, and the first set is used for transmitting excitation radiation from a suitable source to the samples.

17. In a system for measuring epi-fluorescence from samples arranged in a sample plate, the modification that an arrangement of optical fibres for conveying light to a CCD camera is provided, optimised in respect of position and type of said optical fibres for luminescence instead of epi-fluorescence.

1/10

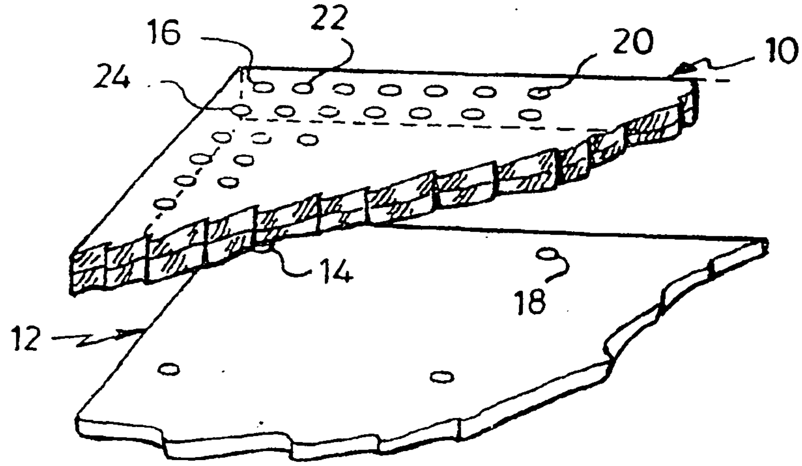


Fig. 1A

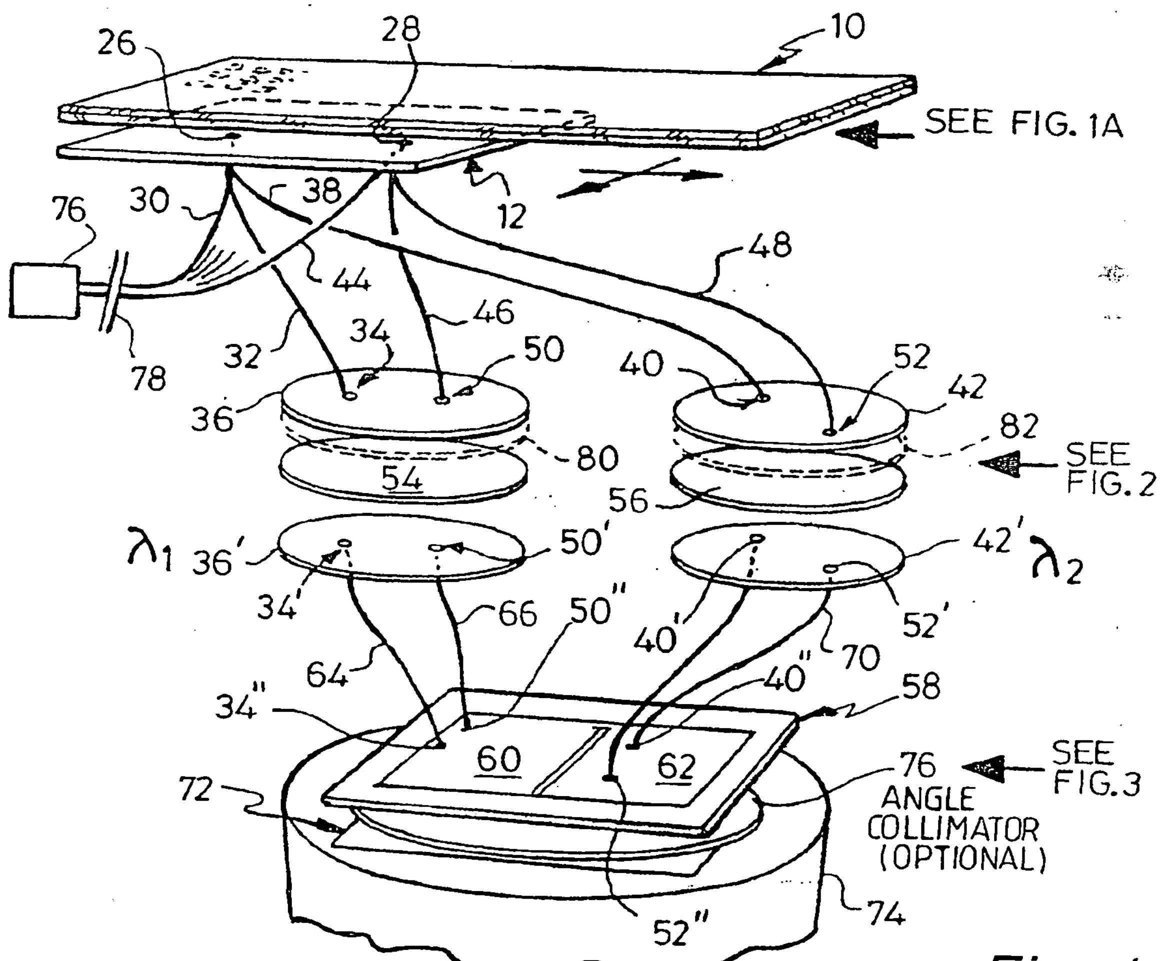
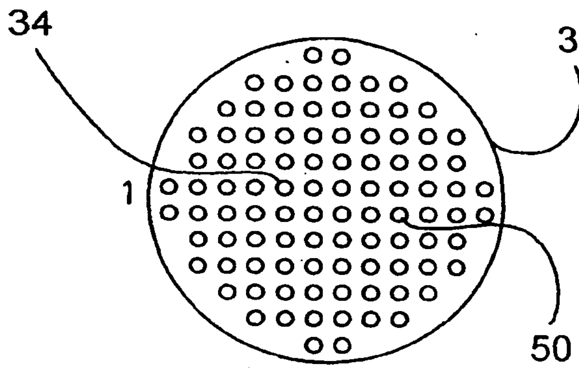
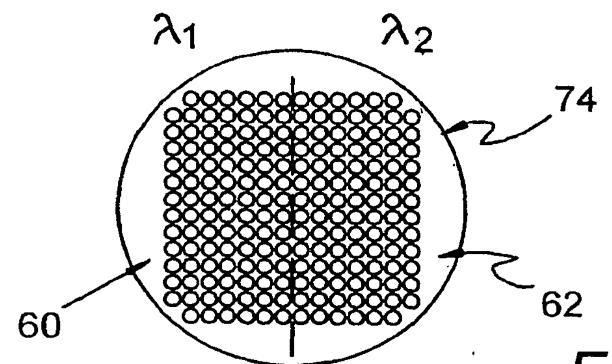


Fig. 1



96 holes
spacing - 3.9mm
in circle - 75mm diameter

Fig. 2



192 holes diameter - 2.0mm
spacing - 1.9mm
within 27 x 27mm
area of camera

Fig. 3

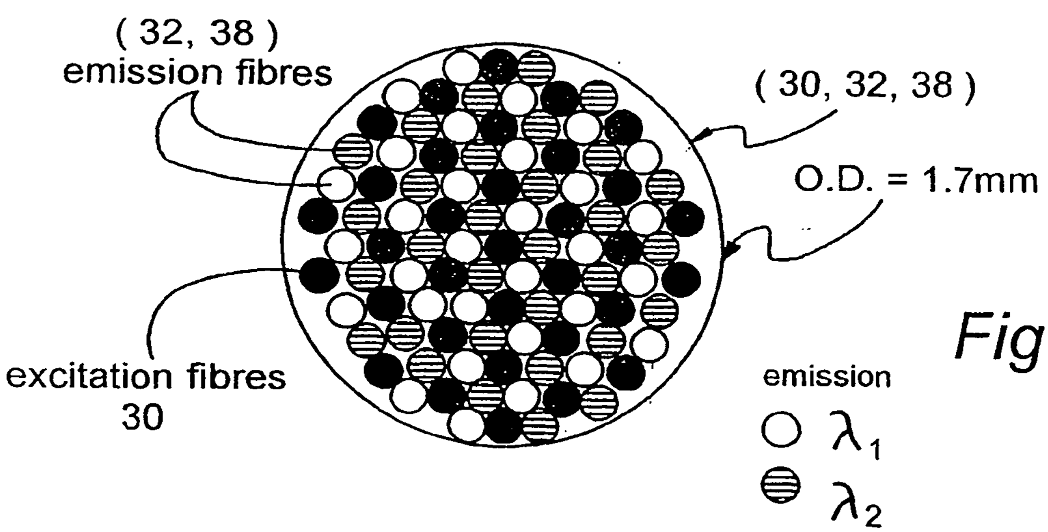


Fig. 4

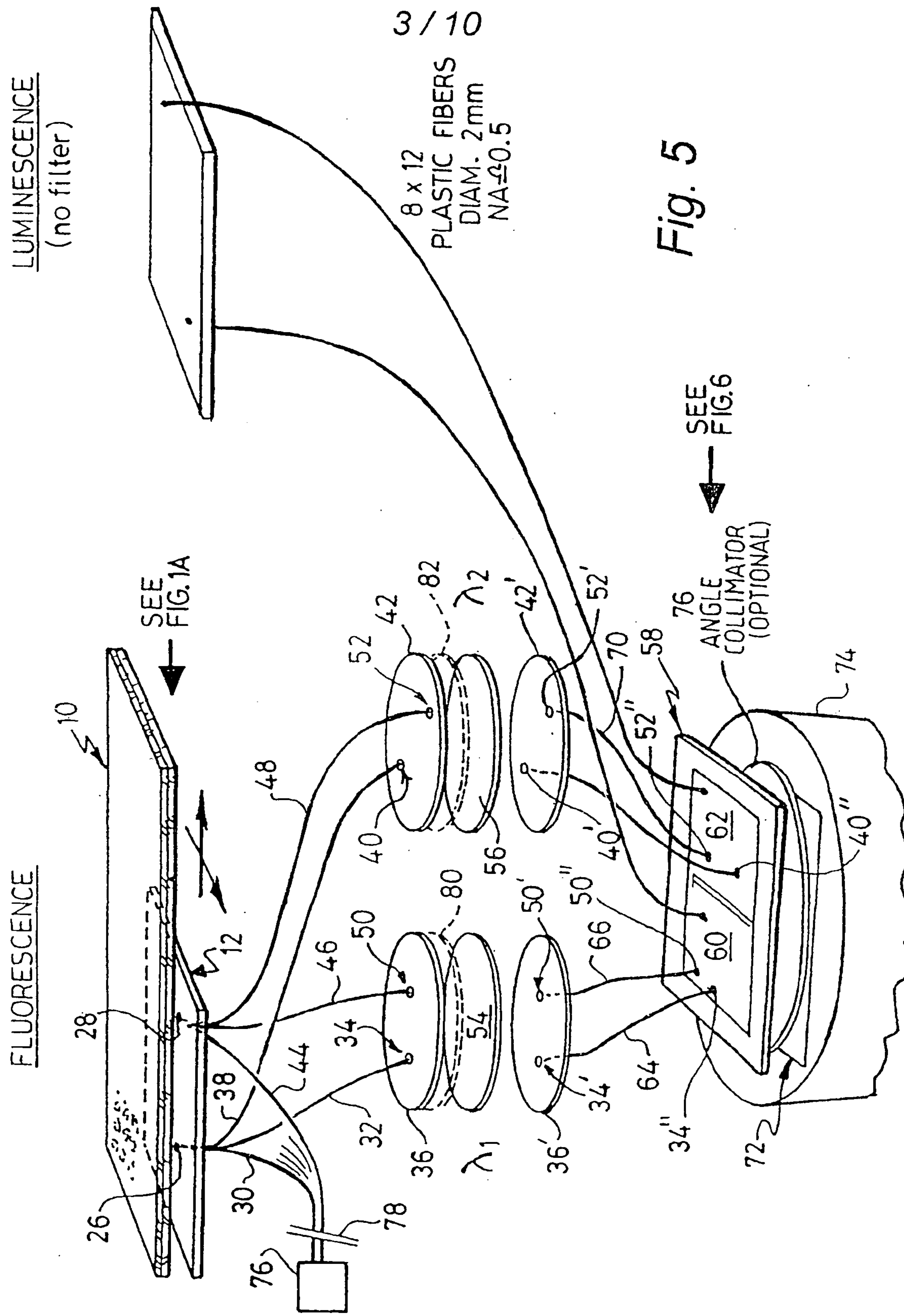

LUMINESCENCE
(no filter)
FLUORESCENCE
8 x 12
PLASTIC FIBERS
DIAM. 2mm
NA≤0.5
SEE
FIG.1A
SEE
FIG.6
ANGLE
COLLIMATOR
(OPTIONAL)

Fig. 5

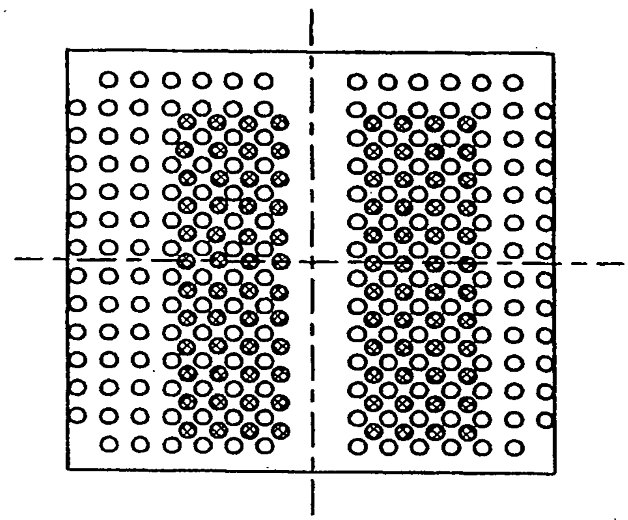


Fig. 6

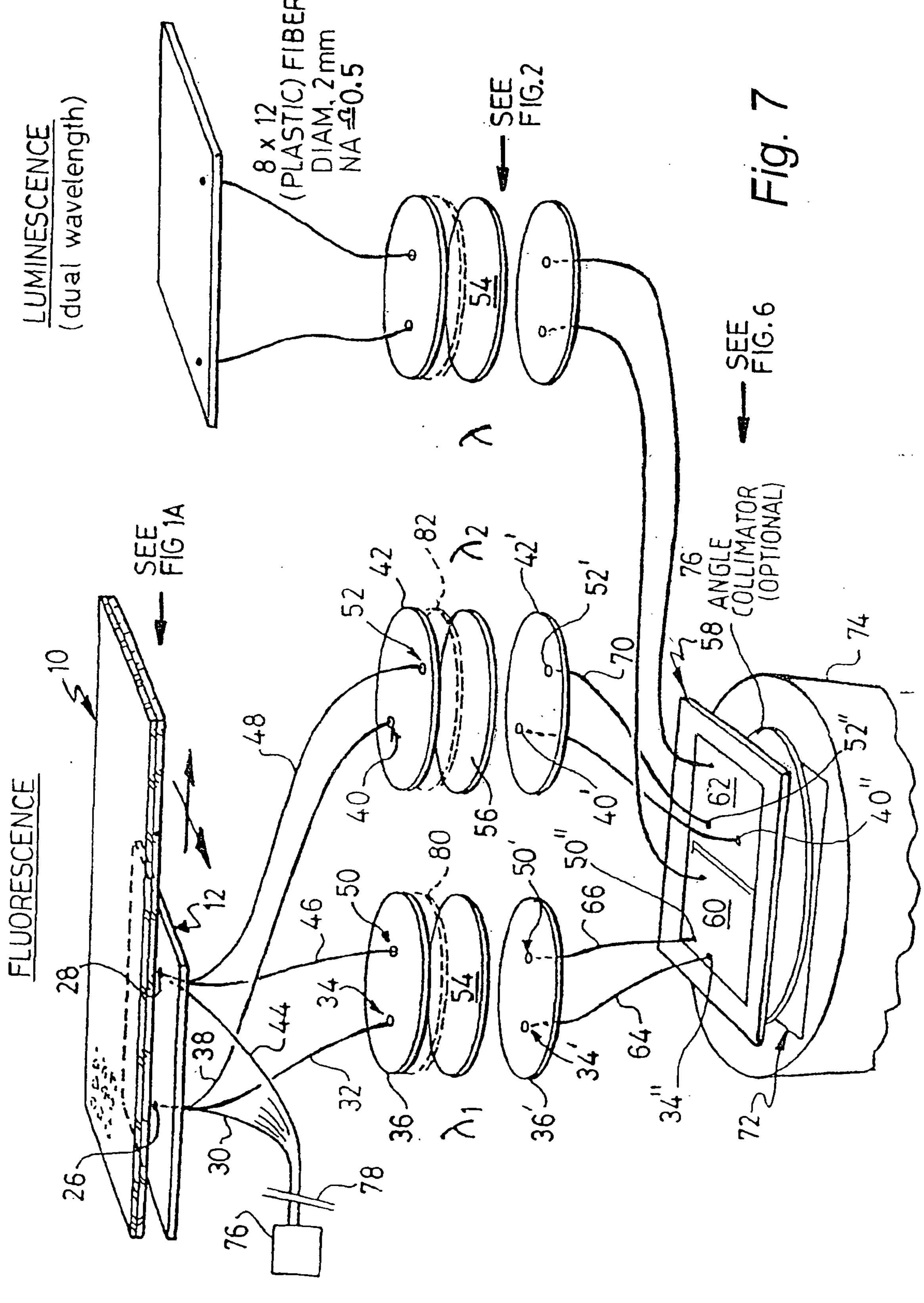

FLUORESCENCE
LUMINESCENCE
(dual wavelength)
8 x 12
(PLASTIC) FIBERS
DIAM. 2mm
NA ≈0.5
SEE FIG.2
SEE FIG.6
SEE FIG 1A
ANGLE
COLLIMATOR
(OPTIONAL)
λ
λ1
λ2
10
12
26
28
30
32
34
34'
34"
36
36'
38
40
40'
40"
42
42'
44
46
48
50
50'
50"
52
52'
52"
54
56
58
60
62
64
66
70
72
74
76
78
80
82

Fig. 7

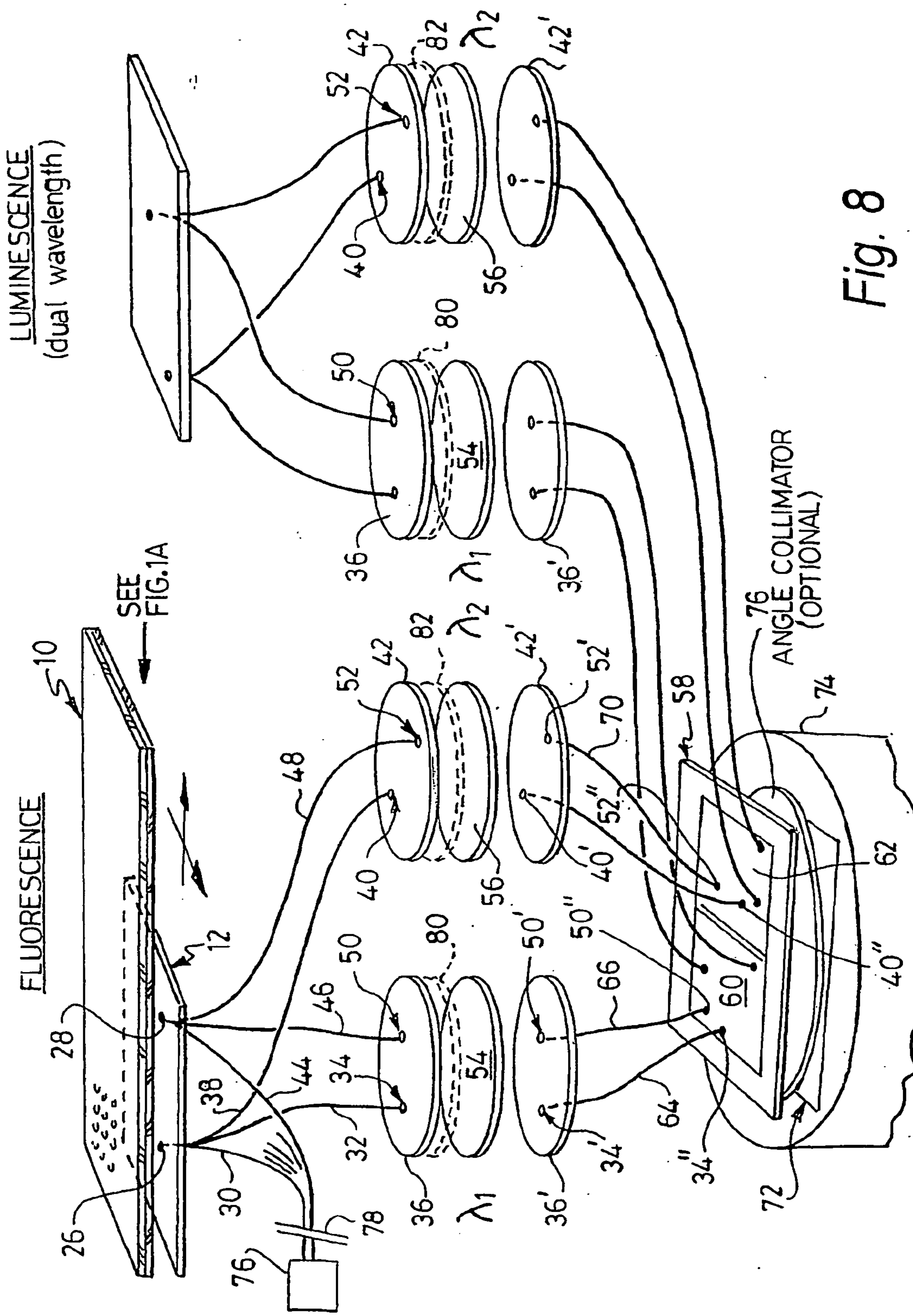

FLUORESCENCE
LUMINESCENCE
(dual wavelength)
SEE FIG.1A
ANGLE COLLIMATOR
(OPTIONAL)

Fig. 8

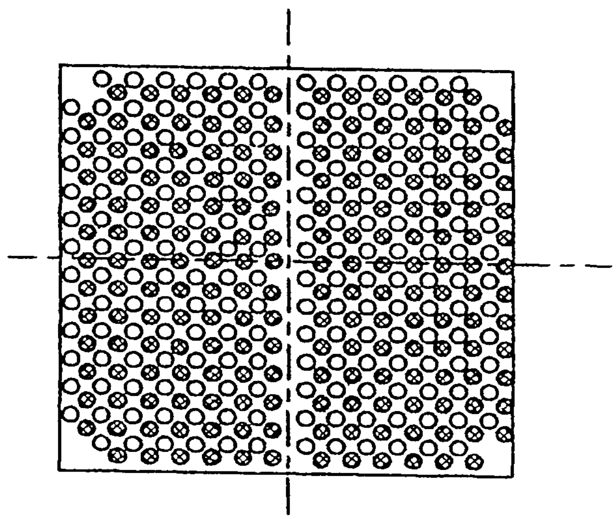
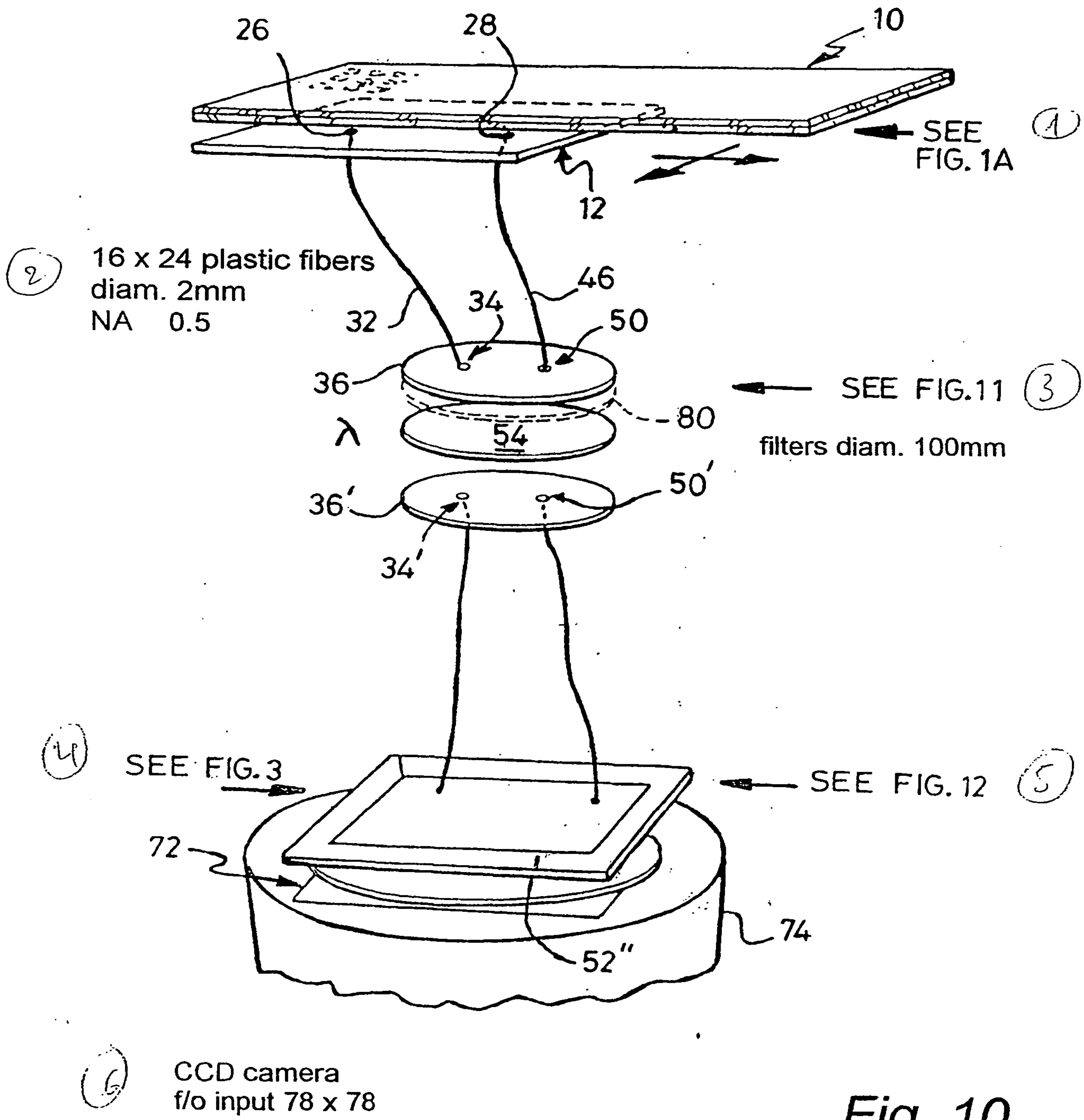


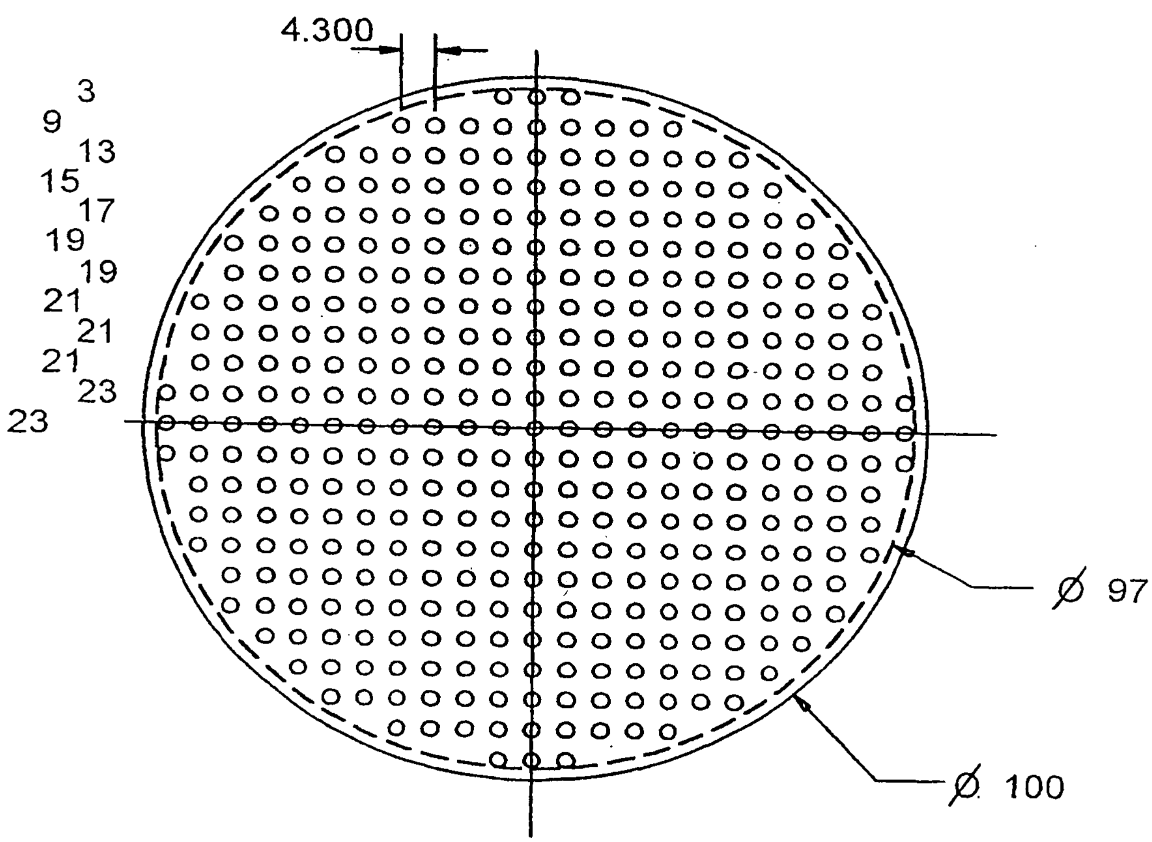
Fig. 9



26
28
10
SEE FIG. 1A
1
12
2
16 x 24 plastic fibers
diam. 2mm
NA 0.5
32
34
46
50
36
SEE FIG.11
3
80
λ
54
filters diam. 100mm
36'
50'
34'
4
SEE FIG. 3
SEE FIG. 12
5
72
74
52''
6
CCD camera
f/o input 78 x 78

Fig. 10

Circles 2mm dia at 4.300 pitch : 100mm dia enclosing circle

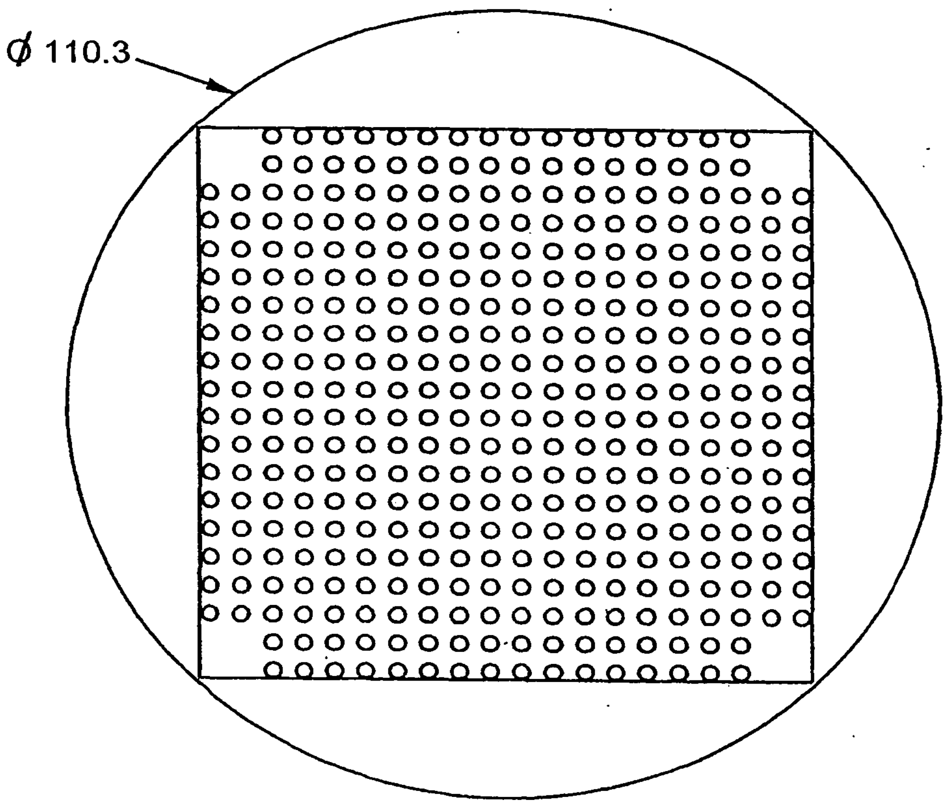


By inspection, No. of 2mm dia. circles within 100mm dia. circle is:

Total = 2 x 181 + 23 = 385

Fig. 11

400 - 384 = 16 , so delete 4 holes from each corner



384 holes diameter 2.0mm
Spacing 4.0mm
within 78 x 78 mm area of camera

Fig. 12

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/05585

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	GB 2 315 130 A (CAMBRIDGE IMAGING LTD) 21 January 1998 (1998-01-21) abstract; claim 8; figures 7,8 ---	1-4, 7-11,17 12
X A	WO 00 05569 A (CAMBRIDGE IMAGING LTD ;HOOPER CLAIRE ELIZABETH (GB); RUSHBROOKE JO) 3 February 2000 (2000-02-03) cited in the application the whole document ---	1-5, 7-11,17 12
X A	WO 00 05568 A (CAMBRIDGE IMAGING LTD ;HOOPER CLAIRE ELIZABETH (GB); RUSHBROOKE JO) 3 February 2000 (2000-02-03) cited in the application the whole document ---	1-5, 7-11,17 12
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
16 April 2002	26/04/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Scheu, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/05585

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 01743 A (CAMBRIDGE IMAGING LTD ;HOOPER CLAIRE ELIZABETH (GB); NEALE WILLIAM) 15 January 1998 (1998-01-15) page 28 -page 29	2-4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 01/05585

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			EP	0910791 A1	28-04-1999
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			WO	9801743 A1	15-01-1998
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